



APPENDIX A
SPECIFICATION / CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE

Applicant: Laura P.W. Ranum, et al.
Serial No.: 09/181,585

SPINOCEREBELLAR ATAXIA TYPE 8 AND METHODS OF DETECTION
Attorney Docket No. 110.00900101

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been bolded.

In the Claims

For the Examiner's convenience, all of the pending claims are shown below.

1. (Twice Amended) A method for detecting the presence of a DNA fragment located within an at-risk allele of an [the] SCA8 coding sequence comprising:
 - (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of two oligonucleotide primers, wherein the SCA8 coding sequence comprises a nucleotide sequence, the complement of which hybridizes to the nucleotide sequence set forth at SEQ ID NO:1 in a hybridization solution at 60°C for 3 hours, followed by two washes for 15 minutes each at room temperature in a solution containing 2x SSC and 0.05 % SDS, followed by two washes for 15 minutes each at 50°C in a solution containing 0.1% SSC and 0.1% SDS;
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 - (b) extending the primers to form complementary primer extension products which act as templates for synthesizing the desired DNA fragment containing the repeat region;
 - (c) detecting the fragment so amplified; and
 - (d) analyzing the amplified DNA fragment for an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

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2. (Amended) A method for detecting the presence of a DNA fragment located within an at-risk allele of the SCA8 coding sequence comprising:

- (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of two oligonucleotide primers [The method of claim 1] wherein a first oligonucleotide primer of the two oligonucleotide primers is chosen from nucleotides 1-448 of SEQ ID NO:1, and a second oligonucleotide primer of the two oligonucleotide primers is chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, wherein each primer has at least 11 nucleotides;
- (b) extending the primers to form complementary primer extension products which act as templates for synthesizing the desired DNA fragment containing the repeat region;
- (c) detecting the fragment so amplified; and
- (d) analyzing the amplified DNA fragment for an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

3. (Amended) A method for detecting the presence of a DNA fragment located within an at-risk allele of the SCA8 coding sequence comprising:

- (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of two oligonucleotide primers [The method of claim 2] wherein the first oligonucleotide primer is selected from the group consisting of SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:4 and wherein the second oligonucleotide primer is selected from the group consisting of SEQ ID NO:6, SEQ ID NO:9, and SEQ ID NO:12;
- (b) extending the primers to form complementary primer extension products

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which act as templates for synthesizing the desired DNA fragment containing the repeat region;

- (c) detecting the fragment so amplified; and
- (d) analyzing the amplified DNA fragment for an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

4. A kit for detecting whether or not an individual has, or is at-risk for developing, spinocerebellar ataxia type 8, the kit comprising a first oligonucleotide primer chosen from nucleotides 1-448 of SEQ ID NO:1, and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, wherein each primer has at least 11 nucleotides, wherein an individual who has or is at risk for developing SCA8 has an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

7. (Amended) A method for detecting the presence of at least one DNA molecule containing a repeat region of an SCA8 coding sequence comprising:

- (a) digesting genomic DNA with a restriction endonuclease to obtain DNA fragments;
- (b) denaturating the DNA fragments to yield DNA molecules and probing the DNA molecules under hybridizing conditions with a detectably labeled probe, which hybridizes to a DNA molecule containing a repeat region of an isolated SCA8 coding sequence, wherein the SCA8 coding sequence comprises a nucleotide sequence, the complement of which hybridizes to the nucleotide sequence set forth at SEQ ID NO:1 in a hybridization solution at 60°C for 3 hours, followed by two washes for 15 minutes each at room temperature in a solution containing 2x SSC and 0.05 % SDS, followed by two washes for 15 minutes each at 50°C in a solution containing 0.1% SSC and 0.1% SDS;

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- (c) detecting the probe which has hybridized to the DNA molecule; and
- (d) analyzing the DNA molecule for a repeat region characteristic of a normal or at-risk form of the SCA8 coding sequence.

8. The method of claim 7 wherein the probe is chosen from nucleotides 1-448 of SEQ ID NO:1 or from nucleotides 726-1,159 of SEQ ID NO:1, or complements thereto, wherein the probe has at least 20 nucleotides.

9. The method of claim 7 wherein the probe comprises nucleotides 19-449 of SEQ ID NO:1, or a complement thereto.

10. (Amended) A kit for detecting whether or not an individual has, or is at-risk for developing, spinocerebellar ataxia type 8 comprising a probe chosen from nucleotides 1-448 of SEQ ID NO:1 or from nucleotides 726-1,159 of SEQ ID NO:1, or complements thereto, wherein each probe has at least 20 nucleotides, wherein an individual who has or is at risk for developing SCA8 has an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

11. (Amended) The method of claim 7 wherein the step of analyzing comprises analyzing for a repeat region comprising at least about 80 CTG repeats.

12. (Amended) The method of claim 7 wherein the step of analyzing comprises analyzing for a repeat region comprising at least about 92 combined CTA and CTG repeats. *E*

13. (Twice Amended) A method for determining whether an individual is not at-risk for developing, spinocerebellar ataxia type 8, the method comprising analyzing a repeat region of a spinocerebellar ataxia type 8 coding sequence wherein individuals who are

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not at-risk for developing spinocerebellar ataxia type 8 have less than 80 CTG repeats in the repeat region or no greater than about 91 combined CTA and CTG repeats in the repeat region, wherein the SCA8 coding sequence comprises a nucleotide sequence, the complement of which hybridizes to the nucleotide sequence set forth at SEQ ID NO:1 in a hybridization solution at 60°C for 3 hours, followed by two washes for 15 minutes each at room temperature in a solution containing 2x SSC and 0.05 % SDS, followed by two washes for 15 minutes each at 50°C in a solution containing 0.1% SSC and 0.1% SDS.

14. (Twice Amended) A method for detecting the presence of a DNA fragment located within an at-risk allele of the SCA8 coding sequence comprising:

- (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of a first oligonucleotide primer pair, wherein the SCA8 coding sequence comprises a nucleotide sequence, the complement of which hybridizes to the nucleotide sequence set forth at SEQ ID NO:1 in a hybridization solution at 60°C for 3 hours, followed by two washes for 15 minutes each at room temperature in a solution containing 2x SSC and 0.05 % SDS, followed by two washes for 15 minutes each at 50°C in a solution containing 0.1% SSC and 0.1% SDS;
- (b) extending the first primer pair to form complementary primer extension products which act as templates for synthesizing a first desired DNA fragment containing the repeat region;
- (c) removing the first desired DNA fragment containing the repeat region;
- (d) treating separate complementary strands of the first desired DNA fragment containing the repeat region with a molar excess of a second oligonucleotide primer pair;
- (e) extending the second primer pair to form complementary primer extension products which act as templates for synthesizing a second desired DNA fragment

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containing the repeat region;

- (f) detecting the second desired DNA fragment so amplified; and
- (g) analyzing the amplified DNA fragment for an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

15. (Amended) A method for detecting the presence of a DNA fragment located within an at-risk allele of the SCA8 coding sequence comprising:

- (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of a first oligonucleotide primer pair [The method of claim 14] wherein the first oligonucleotide primer pair comprises a first oligonucleotide primer chosen from nucleotides 1-448 of SEQ ID NO:1, and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, wherein each primer has at least 11 nucleotides;
- (b) extending the first primer pair to form complementary primer extension products which act as templates for synthesizing a first desired DNA fragment containing the repeat region;
- (c) removing the first desired DNA fragment containing the repeat region;
- (d) treating separate complementary strands of the first desired DNA fragment containing the repeat region with a molar excess of a second oligonucleotide primer pair;
- (e) extending the second primer pair to form complementary primer extension products which act as templates for synthesizing a second desired DNA fragment containing the repeat region;
- (f) detecting the second desired DNA fragment so amplified; and
- (g) analyzing the amplified DNA fragment for an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined

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CTA and CTG repeats in the repeat region.

16. The method of claim 15 wherein the first oligonucleotide primer is selected from the group consisting of SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:4 and wherein the second oligonucleotide primer is selected from the group consisting of SEQ ID NO:6, SEQ ID NO:9, and SEQ ID NO:12.
17. (Amended) **A method for detecting the presence of a DNA fragment located within an at-risk allele of the SCA8 coding sequence comprising:**
 - (a) **treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of a first oligonucleotide primer pair;**
 - (b) **extending the first primer pair to form complementary primer extension products which act as templates for synthesizing a first desired DNA fragment containing the repeat region;**
 - (c) **removing the first desired DNA fragment containing the repeat region;**
 - (d) **treating separate complementary strands of the first desired DNA fragment containing the repeat region with a molar excess of a second oligonucleotide primer pair [The method of claim 14] wherein the second oligonucleotide primer pair comprises a first oligonucleotide primer chosen from nucleotides 449-725 of SEQ ID NO:1, and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, wherein each primer has at least 11 nucleotides;**
 - (e) **extending the second primer pair to form complementary primer extension products which act as templates for synthesizing a second desired DNA fragment containing the repeat region;**
 - (f) **detecting the second desired DNA fragment so amplified; and**
 - (g) **analyzing the amplified DNA fragment for an at-risk allele having at least**

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about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

18. (Amended) A kit for detecting whether or not an individual has, or is at-risk for, developing spinocerebellar ataxia type 8 comprising a first oligonucleotide primer pair comprising a first oligonucleotide primer chosen from nucleotides 1-448 of SEQ ID NO:1, and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, and a second oligonucleotide primer pair comprising a first oligonucleotide primer chosen from nucleotides 449-725 of SEQ ID NO:1, and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, wherein each primer has at least 11 nucleotides, wherein an individual who has or is at risk for developing SCA8 has an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

19. (Amended) A method for detecting the presence of a DNA fragment located within an at-risk allele of the SCA8 coding sequence comprising:

(a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of a first oligonucleotide primer pair;

(b) extending the first primer pair to form complementary primer extension products which act as templates for synthesizing a first desired DNA fragment containing the repeat region;

(c) removing the first desired DNA fragment containing the repeat region;

(d) treating separate complementary strands of the first desired DNA fragment containing the repeat region with a molar excess of a second oligonucleotide primer pair [The method of claim 14] wherein the second oligonucleotide primer pair comprises a first oligonucleotide primer that has three CTA repeats

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followed by three CTG repeats and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1;

- (e) extending the second primer pair to form complementary primer extension products which act as templates for synthesizing a second desired DNA fragment containing the repeat region;
- (f) detecting the second desired DNA fragment so amplified; and
- (g) analyzing the amplified DNA fragment for an at-risk allele having at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.

21. (Twice Amended) An isolated SCA8 coding sequence comprising a repeat region wherein the SCA8 locus is located on the long arm of chromosome 13, and a complement of the coding sequence, wherein the SCA8 coding sequence comprises a nucleotide sequence, the complement of which hybridizes to the nucleotide sequence set forth at SEQ ID NO:1 in a hybridization solution at 60°C for 3 hours, followed by two washes for 15 minutes each at room temperature in a solution containing 2x SSC and 0.05 % SDS, followed by two washes for 15 minutes each at 50°C in a solution containing 0.1% SSC and 0.1% SDS.
22. (Amended) The isolated coding sequence of claim 21 wherein the nucleic acid comprises DNA.
23. The DNA of claim 22 wherein the DNA is cDNA.
24. The DNA of claim 22 wherein the cDNA comprises SEQ ID NO:2.
25. The DNA of claim 22 wherein the cDNA comprises SEQ ID NO:3.

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26. (Amended) The isolated coding sequence of claim 21 wherein the nucleic acid comprises SEQ ID NO:1.
27. An isolated nucleic acid molecule wherein the nucleic acid comprises 1-448 of SEQ ID NO:1, and a complement thereto.
28. An isolated nucleic acid molecule wherein the nucleic acid comprises 726-1,159 of SEQ ID NO:1, and a complement thereto.
29. (Amended) The isolated coding sequence of claim 21 wherein the SCA8 coding sequence comprises nucleotides 1-448 of SEQ ID NO:1 followed by a repeat region.
30. (Amended) The isolated coding sequence of claim 21 wherein the SCA8 coding sequence comprises nucleotides 726-1,159 of SEQ ID NO:1 preceded by a repeat region.
31. An isolated nucleic acid molecule comprising nucleotides 1-448 of SEQ ID NO:1 in a vector.
32. An isolated nucleic acid molecule comprising nucleotides 1-448 of SEQ ID NO:1 and further comprising a repeat region, and a complement thereto.
33. (Amended) An isolated oligonucleotide [comprising] consisting essentially of at least 15 nucleotides from nucleotides 1-448 of SEQ ID NO:1, and the complementary nucleotides thereto. 
34. (Amended) An isolated oligonucleotide [comprising] consisting essentially of at least 15 nucleotides from nucleotides 726-1,159 of SEQ ID NO:1, and the complementary nucleotides thereto.

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35. (Three Times Amended) An isolated oligonucleotide that specifically hybridizes to a nucleic acid molecule comprising a repeat region of an isolated SCA8 coding sequence; the oligonucleotide having at least about 11 nucleotides, wherein the SCA8 coding sequence comprises a nucleotide sequence, the complement of which hybridizes to the nucleotide sequence set forth at SEQ ID NO:1 in a hybridization solution at 60°C for 3 hours, followed by two washes for 15 minutes each at room temperature in a solution containing 2x SSC and 0.05 % SDS, followed by two washes for 15 minutes each at 50°C in a solution containing 0.1% SSC and 0.1% SDS.
36. An isolated recombinant vector comprising the nucleotides of SEQ ID NO:1 operatively linked to heterologous vector sequences.
37. The method of claim 13 wherein individuals who are not at-risk for developing spinocerebellar ataxia type 8 have no greater than about 33 combined CTA and CTG repeats in the repeat region.
38. (Amended) A method for detecting the presence of a DNA fragment located within a not at-risk allele of the SCA8 coding sequence comprising:
 - (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of two oligonucleotide primers, wherein the SCA8 coding sequence comprises a nucleotide sequence, the complement of which hybridizes to the nucleotide sequence set forth at SEQ ID NO:1 in a hybridization solution at 60°C for 3 hours, followed by two washes for 15 minutes each at room temperature in a solution containing 2x SSC and 0.05 % SDS, followed by two washes for 15 minutes each at 50°C in a solution containing 0.1% SSC and 0.1% SDS;
 - (b) extending the primers to form complementary primer extension products which act as templates for synthesizing the desired DNA fragment containing the repeat

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region;

- (c) detecting the fragment so amplified; and
- (d) analyzing the amplified DNA fragment for a not at-risk allele having less than 80 CTG repeats in the repeat region or no greater than about 91 combined CTA and CTG repeats in the repeat region.

39. (Amended) A method for detecting the presence of a DNA fragment located within a not at-risk allele of the SCA8 coding sequence comprising:

- (a) treating separate complementary DNA molecules of a DNA fragment containing a repeat region of the SCA8 coding sequence with a molar excess of a first oligonucleotide primer pair, wherein the SCA8 coding sequence comprises a nucleotide sequence, the complement of which hybridizes to the nucleotide sequence set forth at SEQ ID NO:1 in a hybridization solution at 60°C for 3 hours, followed by two washes for 15 minutes each at room temperature in a solution containing 2x SSC and 0.05 % SDS, followed by two washes for 15 minutes each at 50°C in a solution containing 0.1% SSC and 0.1% SDS;
- (b) extending the first primer pair to form complementary primer extension products which act as templates for synthesizing a first desired DNA fragment containing the repeat region;
- (c) removing the first desired DNA fragment containing the repeat region;
- (d) treating separate complementary strands of the first desired DNA fragment containing the repeat region with a molar excess of a second oligonucleotide primer pair;
- (e) extending the second primer pair to form complementary primer extension products which act as templates for synthesizing a second desired DNA fragment containing the repeat region;
- (f) detecting the second desired DNA fragment so amplified; and
- (g) analyzing the amplified DNA fragment for a not at-risk allele having less than 80

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CTG repeats in the repeat region or no greater than about 91 combined CTA and CTG repeats in the repeat region.

40. (Amended) A method for determining whether an individual [has, or] is at-risk for developing [,] spinocerebellar ataxia type 8, the method comprising analyzing a repeat region of a spinocerebellar ataxia type 8 coding sequence wherein individuals who are at-risk for developing spinocerebellar ataxia type 8 have at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region.
41. (Amended) An isolated nucleic acid molecule wherein a complement of the nucleic acid molecule specifically hybridizes to nucleotides 1-448 of SEQ ID NO:1 under standard hybridization conditions.
42. An isolated nucleic acid molecule wherein a complement of the nucleic acid molecule hybridizes to nucleotides 726-1,159 of SEQ ID NO:1 under standard hybridization conditions.
43. A kit for detecting whether or not an individual is not at-risk for developing spinocerebellar ataxia type 8, the kit comprising a first oligonucleotide primer chosen from nucleotides 1-448 of SEQ ID NO:1, and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, wherein each primer has at least 11 nucleotides, wherein an individual who is not at risk for developing SCA8 has a not at-risk allele having less than 80 CTG repeats in the repeat region or no greater than about 91 combined CTA and CTG repeats in the repeat region. F
44. The kit of claim 43 wherein the number of combined CTA and CTG repeats in the repeat region is no greater than about 33.

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45. A kit for detecting whether or not an individual is not at-risk for developing spinocerebellar ataxia type 8 comprising a probe chosen from nucleotides 1-448 of SEQ ID NO:1 or from nucleotides 726-1,159 of SEQ ID NO:1, or complements thereto, wherein each probe has at least 20 nucleotides, wherein an individual who is not at risk for developing SCA8 has a not at-risk allele having less than 80 CTG repeats in the repeat region or no greater than about 91 combined CTA and CTG repeats in the repeat region.
46. The kit of claim 45 wherein the number of combined CTA and CTG repeats in the repeat region is no greater than about 33.
47. A kit for detecting whether or not an individual is not at-risk for developing spinocerebellar ataxia type 8 comprising a first oligonucleotide primer pair comprising a first oligonucleotide primer chosen from nucleotides 1-448 of SEQ ID NO:1, and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, and a second oligonucleotide primer pair comprising a first oligonucleotide primer chosen from nucleotides 449-725 of SEQ ID NO:1, and a second oligonucleotide primer chosen from nucleotides complementary to nucleotides 726-1,159 of SEQ ID NO:1, wherein each primer has at least 11 nucleotides, wherein an individual who is not at risk for developing SCA8 has a not at-risk allele having less than 80 CTG repeats in the repeat region or no greater than about 91 combined CTA and CTG repeats in the repeat region.
48. The kit of claim 47 wherein the number of combined CTA and CTG repeats in the repeat region is no greater than about 33.
49. (Amended) The method of claim 13 wherein the analyzing comprises sequencing the repeat region of [the] a spinocerebellar ataxia type 8 coding sequence, wherein the

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spinocerebellar ataxia type 8 coding sequence comprises a nucleotide sequence, the complement of which hybridizes to the nucleotide sequence set forth at SEQ ID NO:1 in a hybridization solution at 60°C for 3 hours, followed by two washes for 15 minutes each at room temperature in a solution containing 2x SSC and 0.05 % SDS, followed by two washes for 15 minutes each at 50°C in a solution containing 0.1% SSC and 0.1% SDS.

50. The method of claim 21 wherein the nucleic acid molecule is RNA.
51. The method of claim 7 wherein the analyzing comprises detecting the length of the repeat region.
52. (New) A method for determining whether an individual has spinocerebellar ataxia type 8, the method comprising analyzing a repeat region of a spinocerebellar ataxia type 8 coding sequence wherein individuals who are at-risk for developing spinocerebellar ataxia type 8 have at least about 80 CTG repeats in the repeat region or at least about 92 combined CTA and CTG repeats in the repeat region, and wherein the individual displays at least one symptom of ataxia.